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BLUE STAIN OF COTTON CAUSED BY FUNGI*

O. P. Owen

I. INTRODUCTION

Color has been one of the primary factors affecting the grade of cotton since the first cotton grading standards were established. Its importance has long been recognized by both the growers and buyers of cotton. The price paid the producer for his cotton has often been reduced as much as twenty-five percent because of various spots and stains that have developed in the cotton after it has opened in the field. The most widespread and by far the most important of the stains in reducing the grade is the blue stain which invariably develops in cotton lint that has been exposed in the field.

All varieties of American Upland cotton commonly grown in the state contain a desirable amount of creaminess and luster when the bolls open. When the cotton is picked soon after it has opened it retains this creaminess and luster, but these qualities are lost if it is allowed to remain in the field. The time required for this change to take place and the amount of damage caused by the spots and stains which develop varies from year to year, depending on weather conditions. Excessive rain during the harvest season is especially favorable for the development of spots and stains. The color intensity of the spots and stains is much greater during prolonged rainy periods, while if there is very little rain the spots and stains develop more slowly and the color intensity is not so great.

Some of these color changes may be caused by the cotton coming in contact with the soil, by soot deposited on the lint, by frost injury to the immature bolls, by stains from other parts of the plant, and by fungi and bacteria growing among and in the fibers. Spots and stains of many different colors may be formed by these agencies, but the blue stain is the most common and of greatest economic importance.

The amount of blue stain present in cotton lint varies and the effect it has on the grade of cotton also varies. When the stain has developed so that it gives the bulk of a sample only a grayish tint, or when it has developed in spots, the value of the sample is reduced one grade. However, it is a common occurrence for the stain to develop until the cotton is distinctly blue, which may reduce the value four grades.

Blue stained cotton presents a serious problem to the manufacturer. It must be used in the cheaper grades of cloth, or much more scrubbing and bleaching must be employed before it can be used in manufacturing the better grades of cloth. This additional cost of scrubbing and bleaching blue cotton may be two or three times the cost of bleaching the whiter grades of cotton, but in every case the manufacturer necessarily has to pay a much lower price for the blue-stained cotton.

*These results are taken from a thesis submitted in partial fulfillment for the Master of Science in Plant Pathology. The writer expresses his appreciation for the valuable suggestions of Dr. R. F. Poole in conducting the studies and preparing this bulletin.

REVIEW OF LITERATURE

Comprehensive studies apparently have not been made of the cause or causes of blue or other colors which develop in cotton lint. The literature contains numerous statements indicating that the stains are due to frost, to the lint coming in direct contact with the soil, or to weathering and exposure to the sun.

Mann (11), in 1913, reported a brilliant carmen red discoloration of cotton lint. He stated that the color was due to a fungus of the Fusarium type which had completely invested the fibers and had penetrated the internal cavities of many of them. Jones (8) working in Nigeria described a disease of the cotton plant caused by a species of Alternaria. He reported that two days after hypodermic inoculation of healthy bolls in situ with a spore suspension, the developing lint was tinged red as the result of the formation of a red pigment, which has been claimed to be a morbid symptom of the cotton plant.

A leaf-spot and boll-rot disease of the cotton plant in Rhodesia caused by *Alternaria gossypina* (Thum) was reported by Hopkins (6) in 1931. He stated that boll infection was observed to take place only through wounds and that injury from this type of infection did not appear to be of great significance. However, sooty deposits of spores were found on mature lint when it had been exposed to late rains. This was considered by Hopkins to be of great economic importance inasmuch as an appreciable proportion of the fiber was adversely affected both in color and strength. In another note Hopkins (7) stated that if unseasonable late rains occur after the cotton crop has matured and a majority of the bolls have opened, the lint is commonly infected by mold fungi which cause blackish or grayish discoloration of the fiber.

Bright, Morris and Summers (3) thought the pink and violet colors produced in cotton cloth were caused by species of Fusarium, the colors resulting from the action of the organisms on starch. They suggested that this change might be brought about by the enzymatic action of the organism concerned. These workers also found that the colors of the spots produced were dependent on the nature of the dye as well as the fungus.

Levine and Veitch (10) working on mildew resistance of textile fabrics, found that mildewing of these materials is due to the development of various mold fungi on and in the fabrics. They stated that the simultaneous occurrence of different kinds of molds seemed to play an important part in the development of pink and yellow discolorations in cotton fiber. The fungi seemed to produce a substance having a pink appearance in alkaline or neutral reaction and a yellow one in an acid reaction. Levine (9) in another note advanced the hypothesis that the yellow staining of bleached cotton cloth may be caused by the amount of nitrogenous impurities left in the fiber.

Black, yellow green, brown and red discolorations of cotton cloth were reported by Armstead and Harland (1 and 2). These workers attributed the yellow color to the formation of "crenic acid" by the fungus. Certain species of Penicillium were found to produce this substance. The other colors were caused by a large number of fungi. *Penicillium crustaceum*, *Cladosporium herbarium*, *Epecoccum* sp., *Aspergillus roseus*, *Mucor* sp., *Chaetomium* sp., *Alternaria* sp., and other unidentified fungi. The black

smudges and streaks occurring in the cloth were ascribed to the presence of a large number of spores when some of the fungi were fruiting.

Marsh (12) found a yellow discoloration of the lint in a sample of diseased cotton sent from Nyasaland. He attributed the stain to a fungus and to insects known as cotton stainers. These insects (*Dysdercus* ap.) were reported to puncture cotton bolls and cause staining of lint in Africa. Other workers (4) have shown that when the stainers are bred in the laboratory on food containing no trace of Nemataspora that staining of the lint does not occur when they are allowed to feed on cotton bolls.

STUDIES OF THE CAUSES OF STAIN IN COTTON FIBER

The cause of some of the stains which develop in cotton fiber in the field was made the subject of the study here reported. This work was begun in March, 1936. Cotton that had been exposed all winter and was showing deep blue staining was collected from a number of fields and gin platforms in the Piedmont and Upper Coastal Plain of North Carolina. This cotton was cut into small pieces on sterile microscope slides. These small pieces of fiber were then planted on sterile sweet potato media in Petri plates. The plates were kept at room temperature about 25° C. for about ten days. At the end of this period more than twenty different fungi were obtained from the plates by transferring the mycelium from young colonies to sterile media in Petri plates. Various pigments and various shades of each pigment were produced on the sweet potato media by the different fungi isolated.

After the fungi had been obtained in pure culture, series of experiments were designed to determine, if possible, what effect each might have on cotton fiber. For this purpose clean white cotton treated with distilled water, 1/40, 1/20, and 1/10 molar solutions of dextrose was used. This cotton was sterilized and inoculated with mycelium from the different cultures. It was then incubated for eight weeks at 28° C. After three weeks stains of various colors were produced in some of the cultures. Two stains were outstanding. One was a yellow stain and the other a dark blue, comparing very closely to the blue cotton taken from the field. The fungus causing the yellow stain grew best on the cotton treated with the 1/10 molar dextrose solution, while the one causing the blue stain grew best on the cotton treated with the 1/40 molar solution of dextrose. This indicated that the fungus, an Alternaria type, which caused the blue color was able to grow on the cotton fiber in its natural state more readily than the one causing the yellow stain. Since the blue stain is of greatest economic importance to the producers and manufacturers of cotton, further studies were conducted with the fungus causing it.

The most satisfactory set-up for this work was designed by placing 10 cc. of distilled water in the large test tubes. Small glass tubes were inserted in the test tubes and extended above the surface of the water so the cotton did not come in direct contact with the water. Clean white slivers of mature cotton which had received no treatment except mechanical cleaning to remove pieces of leaves and other foreign material was placed in the tubes. This cotton was sterilized and inoculated with the fungus after which it was incubated at 28° C. Locks containing the seed from practically mature green bolls were also used in these tests. Frequently a slight color change was brought about in the lint of the green locks by sterilization.

Tests were conducted to determine the effect, the state of maturity of the fiber might have on the susceptibility to the attack of the fungus. A large number of green bolls were collected just before they would have opened normally for inoculation studies in the laboratory. These bolls were allowed to open and were immediately picked and ginned. This lint, having a moisture content similar to mature cotton, was placed in quart jars and was inoculated without being sterilized. Mature cotton lint was inoculated in the same way to determine the comparative effect of the fungus on mature and immature fiber.

In order to determine the effect of the fungus on cotton in the bale, the following procedure was employed. Four hundred grams of mature white cotton was weighed and inoculated by placing a culture of the organism grown on cotton in the center of the mass of fiber. After the cotton was inoculated it was compressed until the density was approximately that of a bale compressed by a commercial gin press. These small bales were covered with cheese cloth and tied securely with small wires. A number of bales were stored in the laboratory at ordinary room conditions. Others were placed above the ground in the shed of a tobacco barn. Still other bales were placed above ground on a board out in a cotton field where they were exposed to existing weather conditions.

Field inoculations of mature open bolls and practically mature green bolls were made. The inoculum was introduced into the mature bolls by placing masses of mycelium between the locks of the open bolls. Very small openings were made in the green bolls by prying the carpels apart at the tip of the bolls with a sterile scalpel. A sterile needle was used to insert the pieces of mycelium into the openings. Some of the bolls were inoculated without being surface sterilized, while others had the bracts removed and were surface sterilized by immersing them one minute in a 1-1000 solution of mercuric chloride. After treatment the bolls were washed with sterile water to remove the mercuric chloride left on the surface. A number of different inoculations were made using the procedure just outlined. Half of the bolls in each series were covered with water-proof cellophane bags and the other half were left exposed. While the bags probably did not provide sterile conditions, they did afford a certain amount of protection from external contamination.

Faulwetter (5), Hopkins (6) and Jones (8) have shown that species of Alternaria cause leaf-spots and boll-rots of cotton. In view of the results of these workers, tests were conducted to find out what effect the Alternaria which causes blue stain of the fiber might have on cotton seedlings and more mature plants. Therefore, cotyledonary leaves of seedlings and leaves of older plants were inoculated by placing small pieces of the fungous mycelium on both the lower and on the upper surface of the leaf. A drop of sterile water was placed on the inoculum and covered with a microscope cover glass to retard evaporation.

THE FUNGUS

The fungus is easily isolated by planting spores and infected fiber upon a sweet potato medium. The mycelium when grown on this medium is at first white with a slight greenish tint, turning blue at the center of the colony and soon becomes bluish or grayish black. Different colors are produced on other media. A definite greenish color is formed on corn meal

agar while on oat meal agar the mycelium is white or yellowish. The colors formed on a number of synthetic nutrient media are very similar to those formed on the sweet potato medium.

Conidiophores formed among cotton fibers and on filter paper are olivaceous-brown, septate, and slightly constricted at the septa. They arise singly from vegetative hyphae but occasionally some branching may be observed. The dark brown conidia are formed singly or in chains at the apex of the conidiophores or their branches. They are most frequently without a beak, septate in cross and longitudinal planes, and are slightly constricted at the septa. The conidia are dark brown and vary in size depending on the medium upon which they are grown. On filter paper they are 15 to 63x9 to 20 microns, while those formed on cotton cloth are 15 to 31x12 to 18 microns. Conidia are formed abundantly in three weeks on cotton cloth on a synthetic medium composed of sodium nitrate, dipotassium hydrogen phosphate, magnesium sulphate, potassium chloride, agar, and water. The spores will withstand drying and retain their viability for at least one year.

The Alternaria causing blue stain in cotton fiber definitely differs from those reported by Faulwetter (5), Hopkins (6), and Jones (8) as occurring on other parts of the cotton plant. It is not parasitic on cotton seedling or older plants, causes blue stain in cotton fiber, retains its ability to produce stain for at least two years, has spores most frequently without a beak, and does not form spores on potato dextrose agar. However, it is not the desire of the writer to confuse further the genus Alternaria by proposing a new species. For this reason the fungus is being designed as *Alternaria sp.* as identified by mycologists Fitzpatrick and Martin.

RESULTS OF STUDIES

A. PURE CULTURE INOCULATION STUDIES

1. Mature Fiber

Staining began to appear in the mature cotton in the large test tubes in approximately three weeks after the cotton was inoculated with the fungus (Figure I-A). The first signs of staining were observed on that part of the cotton in contact with the glass tubes. Appearance of the stain in this region was apparently due to a more abundant supply of moisture. The stain continued to develop throughout the whole mass until it was distinctly blue (Figure I-B). The color produced by the fungus was considerably darker than the darkest colored sample in the Universal Standards for American Upland Cotton.

A microscopic examination of the stained cotton revealed masses of dark hyphae ramifying the fibers. Occasionally a hypha could be seen penetrating into the lumen of the fiber, although this was of rather infrequent occurrence. The Alternaria described by Jones (8) penetrated the fiber in a similar way to the one used in this work. When the inoculated cotton was allowed to remain in the tubes for three months or more, a few Alternaria spores could be found. Spore production did not seem to take place until practically all of the water had evaporated from the tubes.

2. Practically Mature Fiber in the Laboratory

Locks from practically mature green bolls began to show definite staining five days after they were inoculated with the fungus. Growth of the fungus was much faster in the immature fiber and the color produced was also

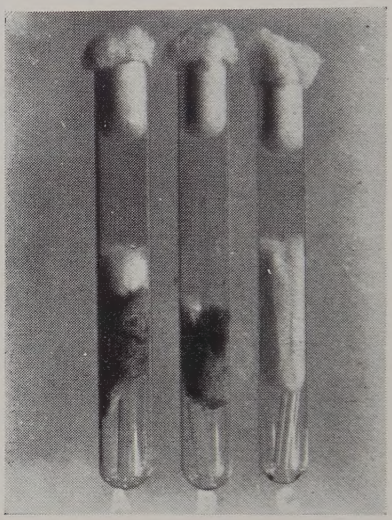

Fig. I.—Inoculated compared with uninoculated cotton. A—Staining first developing. B—The whole mass of cotton completely stained. C—Uninoculated cotton.

darker than that produced on mature fiber. A microscopic examination showed that many more of the immature fibers had been penetrated by hypha. The much higher moisture content of the immature fiber, as well as a higher content of plant sugars, was most likely responsible for the increased growth.

3. Inoculation of Baled Cotton

Sixty-four days after the inoculated bales were stored, one bale from the tobacco barn shed and one from the cotton field were opened. The bale from the shed did not show discoloration of any importance, while the one from the field was uniformly blue throughout. Discoloration was more pronounced and the color intensity was greatest near the place where the inoculum was packed in the bale. The color which developed in this bale was deep enough to cause the cotton to be classed as good ordinary. A few yellow spots appeared in a break or two of the bale which seemed to have been caused by some other organism. A very pronounced musty odor occurred throughout the entire bale.

Other bales which were stored in the laboratory were opened one hundred and seventy-four days after they were stored. Some staining was apparent; however, it was not nearly so deep as that in the bales left in the field. The inner portions of these bales were strict low middling, in color; while the outer portion farthest away from the point where the fungus inoculum was placed graded middling in color. The results obtained in these tests indicate quite clearly that moisture is essential for the best growth of the Alternaria

in stored cotton, yet they also show that the fungus may cause rather severe damage in stored cotton even if it is kept under dry storage conditions.

4. Inoculation of Cotton Bolls in the Field

Definite staining appeared in both the bagged and unbagged bolls thirteen days after the first inoculations were made. No bags were removed at the time of this observation. Staining was particularly noticeable in the second series ten days after the inoculations were made. The greatest color intensity was evident in the locks where the inoculum was placed (Figure II).

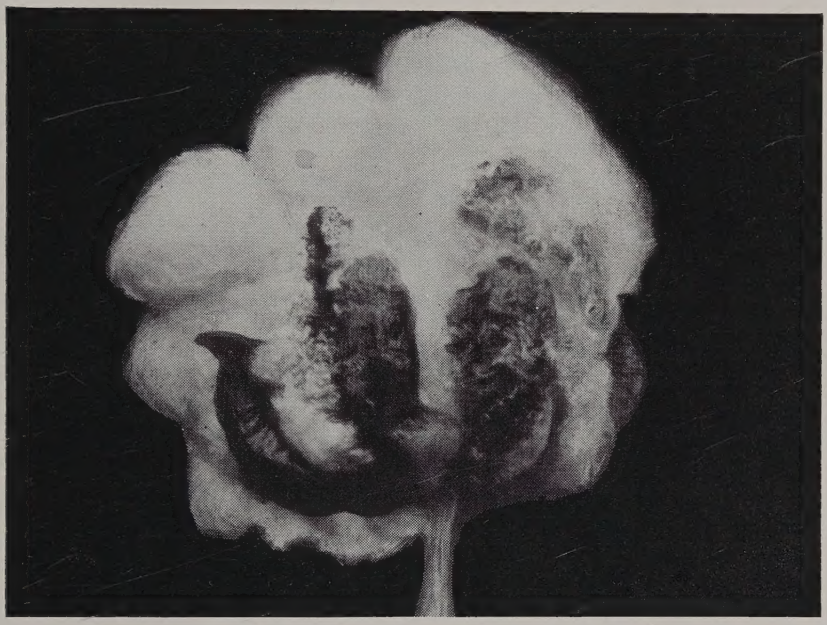

Fig. II.—Cotton boll inoculated in the field showing staining of inoculated lock and adjoining locks after the fungus had spread to them.

In the third series of inoculations very pronounced staining has developed nine days after the inoculations were made. There seemed to be no outstanding difference in the amount and intensity of staining in the bagged and unbagged bolls. Several days of rainy weather occurred while these tests were in progress. During the greater part of the remaining time cloudy weather prevailed. The average maximum temperature during the period was 74° F. and the minimum was 61° F. These temperatures correspond almost exactly with the temperatures favoring growth of the fungus in culture. There seems to be no significant difference in the growth of the fungus at temperatures ranging from 57° to 82° F.

The results obtained compare very closely with those previously obtained when practically mature bolls of several varieties of cotton were inoculated in the field. These bolls were showing considerable staining at the point where the inoculum was placed fifteen days after they were inoculated. At

the time this observation was made the blue color was beginning to appear in other locks as the causative fungus spread to them (Figure II).

When the stain had become very pronounced in the locks into which the causative fungus had spread, a microscopic examination of the stained fibers revealed numerous dark hyphae ramifying the fibers. Spores identical to those of the original culture were found among the fibers. In most cases it was apparent that noticeable deterioration had occurred; however, tests have not been made to determine the total effects on the fiber.

Inoculation of Cotton Seedlings and More Mature Plants

All attempts to infect cotton seedlings growing in the greenhouse and more mature plants in the field failed. Signs of lesions were never observed on any of the inoculated plants. It does not seem that the method of inoculation was faulty, because when the same method was employed using a culture of Alternaria fungus known to produce leaf spot, infection was produced in every case.

DISCUSSION

Cotton has been left in the field to determine the effects of exposure on the color and strength of the fiber. It has also been left in the field to study the relative merits of different methods of harvesting. Before the boll weevil appeared in the state as a serious pest of the cotton plant, a rather large number of relative late maturing varieties of cotton were grown extensively throughout the cotton belt. The bolls on these varieties did not open until late and as a result a rather large portion of the crop was left in the field during the winter. In all of these instances the color of the fiber was adversely affected. Bales of cotton wrapped in cotton cloth bagging have been exposed to find out how well the cotton cloth covering would resist "weathering." It has been quite generally assumed by the growers, the cotton trade, and the manufacturers that colors and stains which developed were caused by frost injury on the plant before the bolls were mature, by soil stains, and by the general condition known as "weathering."

Here, for the first time, the problem of staining of cotton fiber has been approached from a micro-biological point of view. The physiological reactions that take place in the cotton plant may so alter the developing fiber so as to bring about a complete change in the structure and color of the mature fiber. However, after the fiber is mature the possibilities of physiological and metabolic processes causing a change in color are very unlikely, because the mature cotton hair is a dead cell. In view of this, the biological agents most likely to cause staining of the fiber are fungi and bacteria. Most bacteria thrive best on a medium having a high protein content, while most fungi grow best on a medium rather high in carbohydrates. The cotton fiber is almost pure cellulose which makes it a much more favorable medium for the growth of fungi than for bacteria. This, along with the large number of fungi occurring on the cotton plant, without a doubt accounts for much of the staining of the fiber.

Moore and Rankin (13) found that cotton fiber on plants with "rust," the type due to malnutrition, is weak, wasty, and light. They also found that the grade, staple length, fiber thickness, and moisture regain of the fiber are also adversely affected by the disease. It is known that a fungus

of the Alternaria type is universally present on cotton plants suffering from this trouble. In view of the universal presence of the fungus and results of the studies here reported, it seems highly probable that the fungus might be associated with the reduction in grade and strength of the fiber.

From the results of this work it appears quite definitely that many of the so-called "mildew stains" that develop in cotton cloth are due to fungi that occur on the boll, fiber, and other parts of the cotton plant. Since some of these spores will withstand air drying for at least a year and still retain their viability, it is entirely possible that they are carried with the cotton through all of the manufacturing processes and remain in the unsized and unbleached cloth. Some of the sizing materials and bleaching compounds probably contain materials of an antiseptic nature which destroy the viability of the spores in these processes. However, large numbers of spores from the raw cotton are doubtless present in the air in the mill and serve as a constant source of inoculum for the finished goods. If goods infected in either of these ways are stored under conditions of high humidity, "mildewing" is almost certain to occur.

It has been the practice of most workers studying the microbiology of cotton fiber to use various nutrient media to induce the organisms to grow on the fiber. This practice does not appear satisfactory as a method of determining the effect of the organisms on the fiber. There seems to be many organisms that attack cotton fiber when it is placed on a nutrient medium. It is doubtful if they would attack cotton without the addition of the nutrient. For this reason, in these studies cotton fiber in its natural state has been consistently employed for field and culture inoculations. However, it was necessary in most cases to use some method of sterilization. Occasionally a nutrient medium was used in order to hasten the formation of spores.

SUMMARY

1. A discussion of the nature and importance of spots and stains in cotton fiber to the producer and manufacturer is given. It is indicated that losses from blue-stain are of much economic importance.
2. Blue-stain in cotton fiber may be caused by at least one fungus, a species of Alternaria.
3. Immature fiber is more readily attacked by the fungus than mature fiber.
4. This fungus attacks the fiber as the bolls are opening and soon after they have opened.
5. The fungus causes blue stain in baled cotton under different storage conditions.
6. The Alternaria reported here is not parasitic on cotton seedlings and more mature plants.
7. Staining of cotton fiber by this fungus is much greater during periods of high rainfall and excessive moisture.
8. These results indicate that much of the damage caused by blue stain can be avoided by harvesting the cotton soon after the bolls open and by storing under dry conditions.

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